

^{87}Rb NMR studies of the perfused rat heart

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We have studied $^{87}\text{Rb}^+$ fluxes in the perfused rat heart using nuclear magnetic resonance spectroscopy (NMR). A simple model for the interpretation of the data is presented. A comparison of radioactively measured K^+ fluxes and the K^+ fluxes deduced from the $^{87}\text{Rb}^+$ measurements shows them to be very similar. This method provides a means of noninvasively measuring uni-directional K^+ fluxes in the heart.

NMR; Rubidium kinetics; Potassium flux; (Isolated perfused rat heart)

1. INTRODUCTION

NMR measurements of $^{39}\text{K}^+$ have been performed in a variety of tissues [1-6]. The major disadvantage of ^{39}K NMR is its very low sensitivity and the associated poor temporal resolution between successive spectra. As Rb^+ is an established biological congener of K^+ [7], and as the NMR sensitivity of ^{87}Rb is an order of magnitude greater than that of ^{39}K , we have developed ^{87}Rb NMR spectroscopy as a means of measuring K^+ fluxes in cells and perfused organs [8,9].

^{87}Rb is a spin 3/2, quadrupolar nucleus, and as such relaxes very rapidly, giving rise to broad resonance lines. The T_1 and T_2 relaxation times of $^{87}\text{Rb}^+$ in free solution at 25°C are 2.5 ms, corresponding to a resonance of a natural linewidth of 128 Hz. In a 30 g% solution of bovine serum albumin, at 4.2 T, the $^{87}\text{Rb}^+$ linewidth increases to ~450 Hz, while in agarose gels the lines are broader still.

In this paper we present data on the kinetics of $^{87}\text{Rb}^+$ fluxes in the Langendorff perfused rat heart, and their relationship to K^+ fluxes.

2. METHODS

Hearts from 260-290 g male Wistar rats were perfused [10] in the Langendorff mode [11] with Krebs-Henseleit buffer (KHB) [12], modified to contain 1.25 mM CaCl_2 and supplemented with 11 mM D-glucose. Heart rate and coronary flow were monitored throughout. A 30 min stabilization period preceded 60 min baseline KHB perfusion, prior to switching to Rb^+ containing KHB (Rb-KHB) for approx. 150 min. This was followed by 45 min of reperfusion with Rb^+ free KHB. The Rb-KHB had 20% of the KCl replaced with RbCl , resulting in a Rb^+ concentration of 1.33 mM.

^{87}Rb NMR spectroscopy was performed in an Oxford Instruments, vertical bore, 4.2 T magnet, corresponding to a resonant frequency of 59.7 MHz. The heart was hanging freely within an 'earphone' shaped NMR coil, thus eliminating the problems associated with Rb^+ accumulation within any enclosing perfusion chamber. Fully relaxed spectra were acquired with a 90° pulse applied every 12.8 ms. The temporal resolution between successive spectra was 250 s. Quantification of absolute amounts of Rb^+ in hearts was obtained from the ratio of the heart signal to a calibrated reference, shifted 42 ppm downfield, consisting of 1 M RbCl in 5 M KI .

Four spectra were obtained before switching to Rb-KHB, after which spectra were acquired for not less than 90 min. Washout of $^{87}\text{Rb}^+$ from the myocardium was monitored during the Rb^+ free KHB reperfusion. Hearts were weighed at the end of each experiment. Some hearts were freeze clamped, and their absolute Rb^+ content determined by atomic absorption spectrophotometry (AAS). The NMR data were analysed in terms of the following simple kinetic model.

The transport of Rb^+ into the myocardium is assumed to be a result of active and passive uptake and passive efflux. We define I_E and I_I as the extra- and intracellular Rb^+ concentrations, in mM and $\mu\text{mol/g wet wt per min}$, respectively. The ac-

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tive transport, mediated by the ubiquitous transport protein Na-K-ATPase, is assumed to remain at a constant rate of v $\mu\text{mol/g}$ wet wt per min throughout the experiment. The rate constants of passive diffusion into and out of the cells are given by k_{+1} and k_{-1} , respectively. We may thus write

$$\frac{dI_1(t)}{dt} = v + k_{+1}I_E - k_{-1}I_1 \quad (1)$$

The solution of eqn 1, for $I_1(0) = 0$, and the extracellular concentration held constant at $I_E = I_0$, is given by

$$I_1(t) = \frac{v + k_{+1}I_0}{k_{-1}} (1 - \exp(-k_{-1}t)) \quad (2)$$

$(v + k_{+1}I_0)/k_{-1}$ is the equilibrium Rb^+ concentration, I_{max} . In the efflux studies, $I_E = 0$, and thus $v = 0$. If we assume that the concentration just before switching back to KHB is given by I_{max} , then eqn 1 becomes

$$\frac{dI_1(t)}{dt} = -k_{-1}I_1 \quad (3)$$

which has solution

$$I_1(t) = I_{\text{max}} \exp(-k_{-1}t) \quad (4)$$

Eqns 2 and 4 were fitted to all uptake and efflux data.

3. RESULTS

A plot of ^{87}Rb spectra as a function of the duration of Rb-KHB perfusion is shown in fig.1. Each spectrum took 4.2 min to acquire, and was processed with 150 Hz of exponential line broadening. The natural linewidth of the heart ^{87}Rb spectra was ~ 900 Hz. These data, in $\mu\text{mol/g}$ heart tissue, are plotted in fig.2. Table 1 shows the kinetic parameters obtained from the fits of eqns 2 and 4

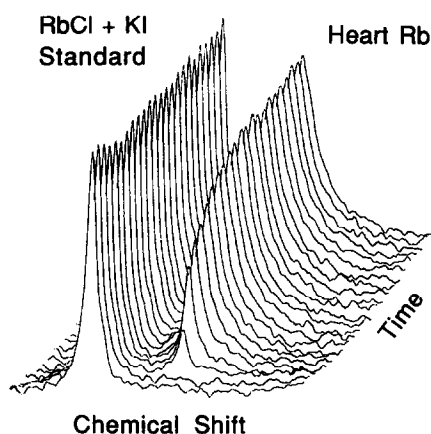


Fig.1. ^{87}Rb NMR spectra of a perfused rat heart as a function of time.

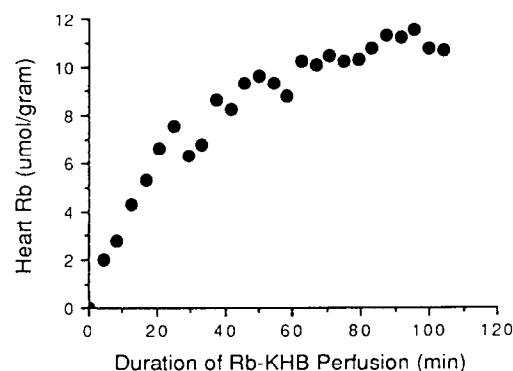


Fig.2. Heart Rb^+ uptake as a function of time.

Table 1

Kinetic parameters for Rb^+ uptake and efflux in the Langendorff perfused rat heart ($n = 5$)

$v + k_{+1}I_0$ ($\mu\text{mol/g}$ per min)	k_{-1}^{uptake} (min^{-1})	k_{-1}^{efflux} (min^{-1})	I_{max} ($\mu\text{mol/g}$)
0.41 ± 0.07	0.035 ± 0.005	0.028 ± 0.002	11.9 ± 1.9

to the uptake and efflux of ^{87}Rb in 5 hearts. The rate constants k_{-1}^{uptake} and k_{-1}^{efflux} are k_{-1} obtained from the uptake and efflux experiments, respectively. Data are presented as mean \pm SD.

Preliminary experiments on the ratio of NMR visible $^{87}\text{Rb}^+$ to Rb^+ determined by AAS showed only 55% ($n = 4$) of the total Rb^+ to be NMR detectable. This result elevated $v + k_{+1}I_0$ to 0.75 $\mu\text{mol/g}$ per min, and I_{max} to 22 $\mu\text{mol/g}$.

The physiological response of the heart to Rb-KHB perfusion is shown in fig.3. Although subject to fluctuation, the heart rate was not substantially altered during Rb-KHB perfusion. Overall, the coronary flow decreased during Rb-KHB perfusion, and failed to recover during KHB reperfusion.

4. DISCUSSION

We have obtained ^{87}Rb NMR spectra from the perfused rat heart with a temporal resolution of ~ 4 min. The simple model proposed explains the observed Rb^+ fluxes, although k_{-1}^{uptake} and k_{-1}^{efflux} are statistically different ($P < 0.025$, Student's t -test), in contrast to the model prediction that they should be identical. We have made no attempt to

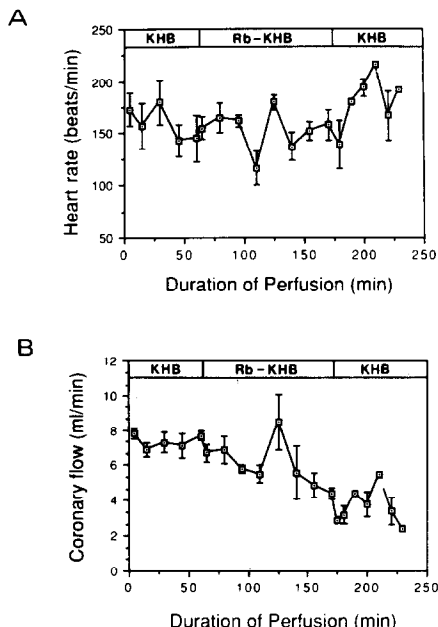


Fig.3. The response of (A) heart rate and (B) coronary flow to Rb-KHB perfusion and KHB reperfusion.

differentiate between intra- and extracellular $^{87}\text{Rb}^+$. However, as the final NMR detectable Rb^+ content of the hearts was $\sim 9 \mu\text{mol}$, and the extracellular content was at most $0.5 \mu\text{mol}$, we are making a tolerable error of $\sim 5\%$ in our measurements. The quadrupolar nature of the ^{87}Rb relaxation implies that the NMR visible $^{87}\text{Rb}^+$ may be between 40 and 100% of the total [13]. Our result of 55% is in agreement with this. We now relate the measured $^{87}\text{Rb}^+$ fluxes to K^+ fluxes. Assuming that the heart transports K^+ and Rb^+ in a similar way, then the K^+ flux into the heart is given by

$$\text{K}^+ \text{ flux} = \frac{(^{87}\text{Rb}^+ \text{ flux}) \cdot (\text{KHB } \text{K}^+ \text{ concentration})}{(\text{Rb-KHB } \text{Rb}^+ \text{ concentration})} \quad (5)$$

This gives a K^+ flux into the heart of $3.75 \mu\text{mol/g}$ per min. Measurements of ^{42}K efflux from the rat heart show the efflux rate to be linearly proportional to heart rate over a range of 15–95 beats/min [14]. Extrapolating to 150 beats/min

gives an efflux rate of $0.038 \cdot \text{min}^{-1}$. Using the accepted value for intracellular heart K^+ concentration of 145 mM, we obtain a K^+ efflux of $5.5 \mu\text{mol/ml}$ cell water per min. We have measured the mass of cell water per g heart tissue to be 0.64. At equilibrium, K^+ uptake and K^+ efflux are, by definition, equal; this gives a uni-directional K^+ influx of $3.53 \mu\text{mol/g}$ per min. The K^+ fluxes calculated from $^{87}\text{Rb}^+$ measurements are in good agreement with those calculated from $^{42}\text{K}^+$ measurements.

This method provides a noninvasive method of determining uni-directional K^+ fluxes in the heart, and other organs.

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